Solid-Phase Extraction

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Polycyclic aromatic hydrocarbons (PAHs) are usually defined as a group of chemicals with two or more fused benzenoid rings. If elements other than carbon and hydrogen are present, the term polycyclic aromatic compounds (PAC) is used. PAHs are natural constituents of crude oil and many other petrochemical products and they are also formed by the incomplete combustion of organic matter. Therefore, they enter the environment from a wide variety of sources such as automobile emissions, industrial processes, waste incineration facilities, domestic heating systems and natural events such as forest fires and volcanic eruptions.

Natural sources of PAH emission have always existed but since the industrial revolution there has been a rapid increase in the loading of the environment with petrochemical PAHs and particularly with PAHs of pyrolytic origin.

The PAHs, both from natural and anthropogenic origins, are released into the atmosphere as vapour or adsorbed on to particles. They are then dispersed and transported long distances by wind before being deposited on the ground or in water, directly or in rain. PAHs are also present in water samples as a result of sewage from industries which use these compounds in their manufacturing processes (e.g. pharmaceuticals, explosives, plastics, synthetic dyes, pesticides and so on).

PAHs are one of the most contaminating groups of pollutants in the environment because of their mutagenic and carcinogenic activity. The activity of each compound depends on its chemical structure. Typically, environmental samples contain a complex mixture of various PAHs, including isomeric structures and both alkylated and nonalkylated PAH forms. **Figure 1** shows the structure of the 16 PAHs considered as priority pollutants by the US Environmental Protection Agency (EPA).

In PAH mixtures of petrochemical origin, two- or three-ring compounds are more abundant than the heavier PAHs, which have four or more rings. On the other hand a typical mixture from a high temperature combustion source contains mainly unsubstituted compounds and PAHs with four or more rings are more abundant than the smaller ones. So, PAHs in natural samples are always encountered as mixtures.

In the environment, PAHs may be eliminated or transformed to even more toxic compounds by chemical reactions such as photooxidation, sulfonation or nitration. For instance, pyrene, which is not mutagenic, can be transformed to nitropyrene, which is highly mutagenic, when nitrogen dioxide is present. In some conditions, traces of nitric acid in the nitrogen dioxide can also transform some PAHs into nitro-PAHs.

Because of their mutagenic and carcinogenic properties, PAHs have been determined in a variety of environmental matrices including air, water, soil, sludge and tissue. The European Community directive (98/83/EC) on the quality of drinking water established the global maximum admissible concentration of four PAHs (benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, benzo(*ghi*)perylene and indeno (1,2,3,*c*-*d*)pyrene) as 0.10 $\mu g\,L^{-1}$ and specified an individual value of 0.010 $\mu g\,L^{-1}$ for benzo(*a*)pyrene. The World Health Organization (WHO) considers only benzo(*a*)pyrene with a reference value of 0.7 $\mu g\,L^{-1}$.

PropBooks

Determination of PAHs

PAHs can be determined by high performance liquid chromatography (HPLC), gas chromatography (GC), supercritical fluid chromatography (SFC) and capillary electrophoresis (CE), but the preferred method is reversed-phase HPLC.

In GC, the most usual detection systems are flame ionization and mass spectrometry, with the latter becoming increasingly common. However, GC is unable to determine the nonvolatile and high molecular mass PAHs. SFC has also been used; its main advantages are its high separation efficiencies and short analysis times. UV-visible, fluorescence or mass spectrometric detectors can all be used.

Micellar electrokinetic capillary chromatography (MEKC) or cyclodextrin-modified CE are capable of separating all 16 PAHs in under 20 min. Despite the advantages in resolution power, the main drawback of MEKC is its poor concentration sensitivity, but this can be partially solved by using a fluorescence detector.

HPLC with a diode array detector enables the UV-visible spectra to be compared and is quite often used to determine PAHs. Fluorescence detection is the most suitable because of its higher sensitivity, although a few PAHs – for instance, acenaphthylene – do not show fluorescence. Spectrofluorimetric

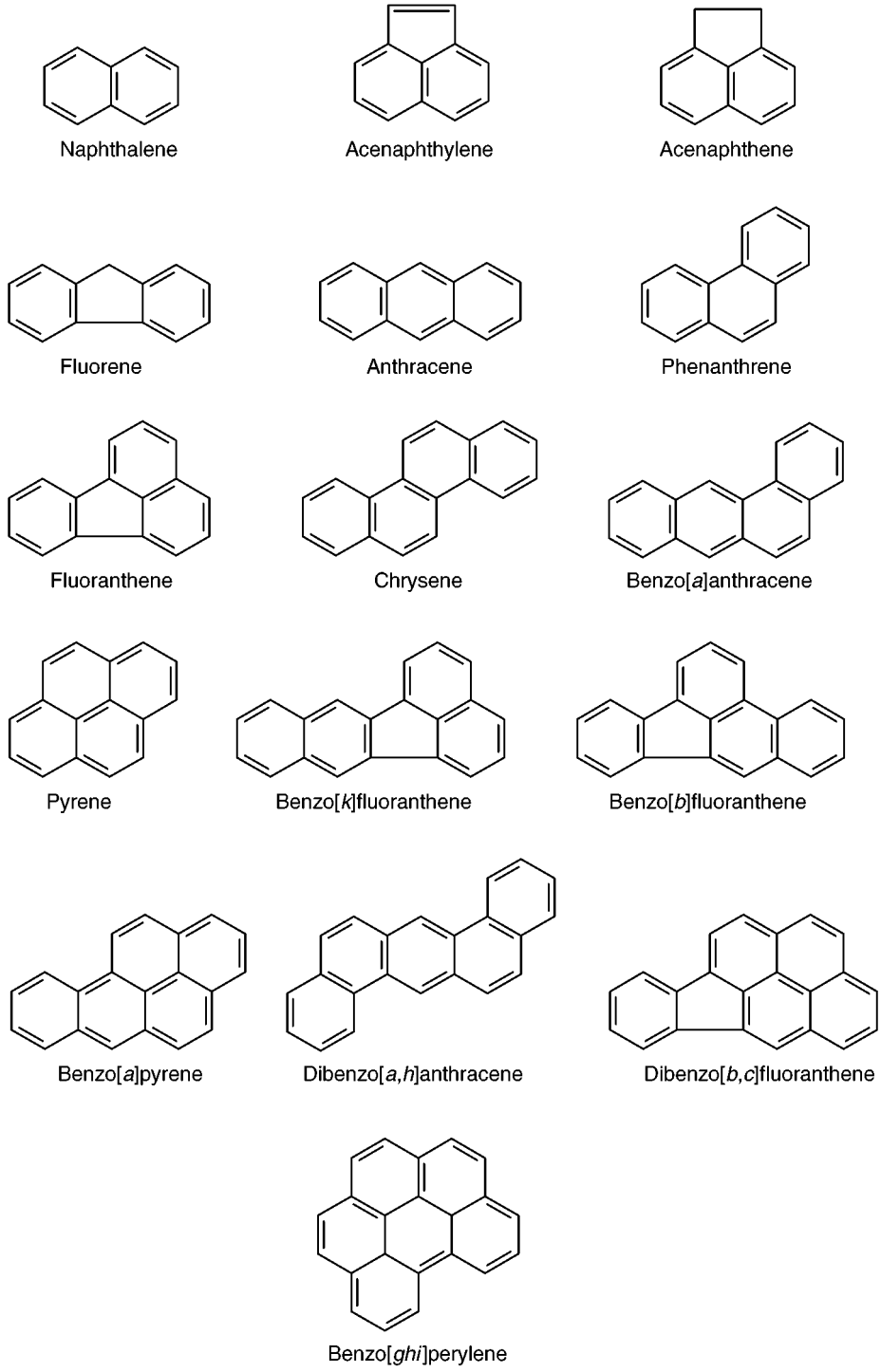

Figure 1 Chemical structure of the 16 PAHs considered to be prioritary pollutants by the US EPA.

detection and wavelength programming enable each compound to be detected at its maximum emission wavelength and so sensitivity and selectivity can be improved. If a micellar mobile phase is used instead of an aqueous–organic one, lower concentrations can be detected. Other detection techniques, such as amperometry, have also been used and the limits of detection are lower than those obtained with UV detection.

Sample-handling Techniques

Because of their toxicity and the low levels to be determined, PAHs are determined in various kinds of

samples which usually require treatment before chromatographic determination. For solid samples, such as soils, sludge, sediments and tissues, the classical Soxhlet extraction is still used, but the extraction is tedious and time-consuming. Other newer techniques, such as supercritical fluid extraction, microwave-assisted solvent extraction and ultrasonic extraction, have proved to be suitable for extracting PAHs and the times required are considerably less. Some of these techniques are followed by a clean-up step, usually performed by solid-phase extraction.

For gaseous samples, different kinds of adsorbents are used to retain the PAHs. PAHs associated with particulate matter are usually collected on filters and then vaporized and PAHs in the vapour phase are trapped by a back-up solid sorbent. Samples are desorbed using Soxhlet apparatus, ultrasonication or supercritical fluid extraction. Sample clean-up is usually required and performed by solid-phase extraction.

Liquid–liquid extraction is gradually being replaced as a technique for extracting organic compounds from liquid samples because it uses considerable amounts of toxic solvents. In this respect, solid-phase extraction has been shown to give good results. Other techniques, such as closed-loop stripping or supercritical fluid extraction, alone or after the solid-phase extraction of the aqueous sample, also give good results. For water samples, results are better when supercritical fluid extraction is applied after solid-phase extraction and not when supercritical fluid extraction is applied alone.

DropBooks

Solid-phase Extraction

In the determination of PAHs, solid-phase extraction is mainly applied to liquid samples but, as already mentioned, it is also applied in the clean-up step with gaseous or solid samples after other extraction techniques. The following discussion mainly refers to the analysis of aqueous samples but some references will be made to other kinds of samples.

Several kinds of sorbents in a variety of devices (e.g. cartridges or discs) may be used for the solid-phase extraction of PAHs. Solid-phase extraction is usually carried out in the offline mode but the online mode does have some advantages and recently has been increasingly used. Solid-phase extraction has been coupled to HPLC and also to GC and SFC to determine PAHs.

One of the most recently developed techniques for extracting PAHs is solid-phase microextraction (SPME) where the sorbent is coated on to a fused-silica fibre mounted in a syringe-like device. SPME is a solvent-free technique, which is its main advantage, but it also has disadvantages compared to SPE.

Addition of Solvent or Micellar Media to Aqueous Samples

The solid-phase extraction of PAHs may seem to be a simple process because of their high hydrophobicity and because there are no expected breakthrough problems. However, the low solubility of the PAHs, which decreases as molecular weight increases, does present a problem. There may also be sorption problems during sampling and storage and to avoid these problems, it is necessary to increase their solubility. Normally this is done by adding organic solvents, such as acetonitrile, methanol and 2-propanol, or surfactants to the sample as solubilizers. The percentage of this solvent in the samples is a critical parameter because if it is low it may not be enough to solubilize the high molecular weight PAHs, whereas if it is high, the breakthrough volume will be low for the low molecular weight PAHs. Therefore, for each kind of sample, solid-phase extraction sorbent and organic solvent, this parameter must be optimized. Typical values are between 10 and 25%, depending on the solvent added and the sorbent used. **Table 1** shows the influence of 2-propanol on the PAH recoveries. The higher the percentage of 2-propanol, the lower the recovery of low molecular weight PAHs, whereas the higher the percentage of 2-propanol, the higher the recoveries of high molecular weight PAHs.

Surfactants have also been used instead of organic solvents. Surfactant molecules are amphiphilic, and have a polar and nonpolar moiety. Because of their amphiphilic nature, surfactant molecules can dissolve in water as monomers, or form a micelle with other surfactant molecules. The concentration at which they start to form micelles is termed the critical micelle concentration (CMC). The CMC depends on factors such as the surfactant itself, temperature, ionic strength and the presence of organic additives. It is the concentration at, or concentration range over which, solution properties like surface tension show an abrupt change in value. To solve the problems arising from the low solubility of PAHs in water and their adsorption on surfaces, surfactants such as sodium dodecyl sulfate (SDS; anionic type, molecular mass 288 and 8.3×10^{-2} mol L^{-1} CMC), cetyl trimethylammonium chloride (CTACl; cationic type, molecular mass 320 and 8.0×10^{-4} mol L^{-1} CMC), Brij-35 (neutral type, molecular mass 1182 and 1.0×10^{-4} mol L^{-1} CMC), have been used, among others. The concentration of surfactant significantly affects the recovery of the PAHs, as shown in **Table 2**, where the influence of the surfactant concentration on PAH recovery is demonstrated.

Table 1 Influence of the percentage of 2-propanol in a sample of PAHs using C_{18} and SDB membrane extraction. (Results are the mean of three determinations)

Compound	*Recovery and RSD (%) in the presence of 2-propanol*					
	0%		*10%*		*15%*	
	C_{18}	*SDB*	*C_{18}*	*SDB*	*C_{18}*	*SDB*
Naphthalene	86 ± 3.1	89 ± 3.0	80 ± 5.2	75 ± 7.6	70 ± 7.2	62 ± 8.7
Acenaphthylene	83 ± 4.3	85 ± 3.1	80 ± 5.5	80 ± 4.5	75 ± 6.1	65 ± 7.6
Acenaphthene	85 ± 3.3	87 ± 3.6	83 ± 4.5	82 ± 4.3	81 ± 5.6	70 ± 6.3
Fluorene	87 ± 3.5	85 ± 4.1	85 ± 3.5	80 ± 5.5	86 ± 5.8	73 ± 6.3
Phenanthrene	84 ± 4.0	88 ± 3.2	92 ± 3.0	90 ± 3.2	95 ± 3.2	78 ± 5.7
Anthracene	79 ± 5.2	81 ± 3.1	85 ± 3.4	75 ± 4.5	93 ± 3.1	78 ± 5.5
Fluoranthene	75 ± 5.0	70 ± 4.9	92 ± 3.1	80 ± 4.8	97 ± 3.0	79 ± 4.8
Pyrene	74 ± 6.1	70 ± 5.2	92 ± 3.2	75 ± 5.6	97 ± 3.3	71 ± 5.8
Benzo(*a*)anthracene	64 ± 7.5	52 ± 7.6	72 ± 4.6	82 ± 4.7	98 ± 3.4	85 ± 4.3
Chrysene	71 ± 6.8	53 ± 8.2	75 ± 5.2	80 ± 3.5	99 ± 3.2	84 ± 3.8
Benzo(*b*)fluoranthene	66 ± 7.4	45 ± 8.7	74 ± 6.3	77 ± 4.7	99 ± 3.1	85 ± 4.1
Benzo(*k*)fluoranthene	74 ± 6.9	50 ± 7.8	85 ± 4.1	78 ± 5.6	95 ± 3.2	88 ± 4.0
Benzo(*a*)pyrene	54 ± 8.7	44 ± 8.6	75 ± 5.2	69 ± 7.5	95 ± 4.0	85 ± 4.2
Dibenz(*a,h*)anthracene	59 ± 8.8	39 ± 8.9	77 ± 5.6	73 ± 7.7	97 ± 3.3	72 ± 5.6
Benzo(*ghi*)perylene	60 ± 8.3	37 ± 10.0	81 ± 4.8	70 ± 8.3	97 ± 3.1	82 ± 4.8
Indeno(*1,2,3-cd*)pyrene	63 ± 7.9	40 ± 9.4	90 ± 3.3	75 ± 8.0	93 ± 3.1	80 ± 6.2

SDB, styrene-divinylbenzene. RSD, relative standard deviation.

Sorbents

For aqueous samples, C_{18}-bonded silica is mainly applied, although good results can also be obtained with styrene-divinylbenzene (SDB). Some authors claim that results are best when different sorbents are combined. For example, in the analysis of drinking water samples, the best results are obtained from combined octadecylsilane (C_{18})–ammonia (NH_2) solid-phase cartridges, whereas PAHs from soil samples were best extracted with silica (Si)–cyano (CN) or C_{18}–CN combinations.

Table 2 Influence of surfactant concentration on recovery of PAHs, after 10 mL preconcentration on a 10 × 3 mm i.d. Boos silica pre-column

Compound	*Analyte recovery (%; n = 2) at Brij-35 concentration ($\times 10^{-4}$ mol L^{-1}) of*				
	0.0	*0.5*	*1.1*	*3.0*	*6.0*
Naphthalene	18	15	13	12	12
Acenaphthene	55	53	55	39	32
Fluorene	73	75	83	54	38
Phenanthrene	42	37	52	84	75
Anthracene	41	38	55	80	69
Fluoranthene	21	23	48	91	84
Pyrene	17	20	46	99	88
Benzo(*a*)anthracene	16	20	46	94	64
Chrysene	16	19	45	94	62
Benzo(*b*)fluoranthene	14	26	49	82	54
Benzo(*k*)fluoranthene	14	23	48	81	54
Benzo(*a*)pyrene	14	24	49	81	52
Dibenz(*a,h*)anthracene	11	30	53	62	31
Benzo(*ghi*)perylene	7	29	32	50	31
Indeno(*1,2,3-cd*)pyrene	7	28	32	50	31

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One group of researchers compared three different bonded silica sorbents, C_{18}, cyano and phenyl, and demonstrated that results are best for C_{18}-bonded silica. Results are shown in **Table 3**, where CH_3CN and CH_2Cl_2 are used as organic solvents to elute PAHs.

The chromatographic system to be used must also be taken into account. It has been demonstrated that, to determine PAHs by HPLC–fluorescence in lake sediments, C_{18}-bonded silica and silica columns could be used satisfactorily in the clean-up and preconcentration step. However, they could not be used with GC–mass spectrometry (GC-MS) for PAHs greater than chrysene, due to interference from aliphatic waxes.

Another group tested various specific sorbents such as Chromspher Π, a diol-treated porous glass chemically modified with a copper phthalocyanine trisulfonic acid derivative (Boos glass), a diol-modified silica with the same copper compound (Boos silica) and a copper phthalocyanine trisulfate-modified polymethacrylamide. It was shown that the second material (Boos silica) was quite useful for the trace enrichment of PAHs containing three or more fused benzene rings but for the smaller PAHs, conventional C_{18}-bonded silica gave better results.

Table 3 Comparison of recoveries for different solid phases with CH_2Cl_2 or CH_3CN

PAH	Octadecyl phase				Cyano phase				Phenyl phase			
	CH_2Cl_2		CH_3CN		CH_2Cl_2		CH_3CN		CH_2Cl_2		CH_3CN	
	%Recovery	%RSD	%Recovery	%RSD	%Recovery	%RSD	%Recovery	%RSD	%Recovery	%RSD	%Recovery	%RSD
1	108	7	71	29	32	14	19	32	72	3	45	22
2	97	3	67	32	57	12	30	27	81	12	68	11
3	112	3	71	39	82	8	34	19	90	11	64	20
4	109	4	68	50	96	7	39	31	90	12	54	21
5	23	6	57	66	94	14	38	54	88	7	35	9
6	85	6	53	73	88	16	36	64	80	6	31	12
7	73	11	46	89	73	35	28	86	105	3	23	8
8	72	13	45	95	73	34	27	90	105	2	22	10
9	51	13	46	57	44	41	38	54	65	11	40	31
10	63	10	54	34	49	40	45	39	66	11	51	19
11	53	12	50	68	46	49	44	46	60	23	48	29
12	27	12	55	53	46	15	42	54	60	16	52	30
13	57	18	47	75	49	27	46	47	60	25	47	54
14	61	13	61	57	58	26	58	39	62	36	60	22
15	53	26	53	60	51	31	51	51	56	45	54	33
16	57	19	56	81	52	33	51	36	59	32	57	36

Volatile PAHs: 1, naphthalene; 2, acenaphthylene; 3, acenaphthene; 4, fluorene; 5, phenanthrene; 6, anthracene; 7, fluoranthene; 8, pyrene. High molecular PAHs: 9, benz(*a*)anthracene; 10, chrysene; 11, benzo(*b*)fluoranthene; 12, benzo(*k*)fluoranthene;13, benzo(*a*)pyrene; 14, indeno(*1,2,3-cd*)pyrene; 15, dibenz(*a,h*)anthracene; 16, benzo(*ghi*)perylene.
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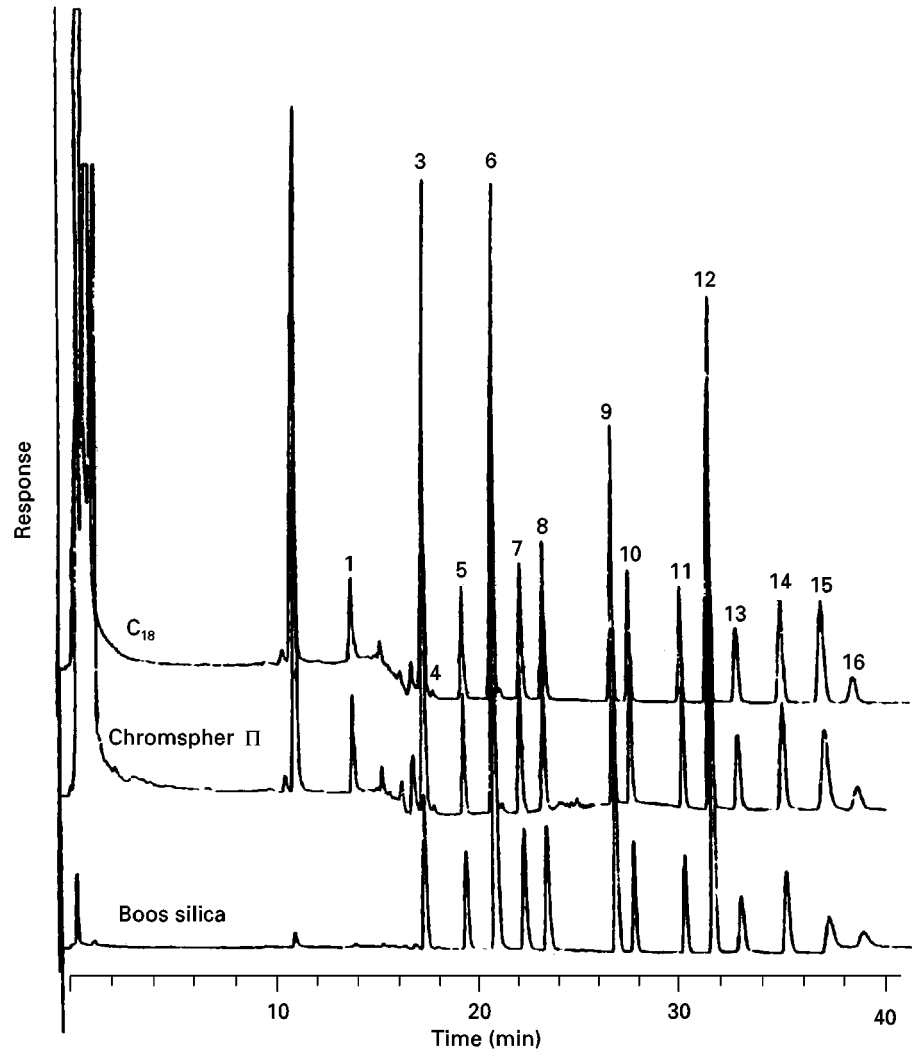

Figure 2 Online trace enrichment LC–fluorescence chromatograms of 10 mL surface water, containing 3×10^{-4} mol L^{-1} Brij-35, spiked at the 100 ng L^{-1} level with all PAHs, using Boos silica, Chromspher Π or C_{18}-bonded silica pre-columns. Peak assignation: 1, naphthalene; 2, acenaphthene; 3, fluorene; 4, phenanthrene; 5, anthracene; 6, fluoranthene; 7, pyrene; 8, benzo[*a*]anthracene; 9, chrysene; 10, benzo[*b*]fluoranthene; 11, benzo[*k*]fluoranthene; 12, benzo[*a*]pyrene; 13, dibenz[*a,h*]anthracene; 14, benzo[*ghi*]perylene; 15, indeno[*1,2,3-cd*']pyrene. For other conditions, see text. Reproduced with permission from Brouwer *et al.* (1994).)

Figure 2 shows chromatograms obtained in the analysis of 10 mL of surface water spiked with PAHs using Boos silica, Chromspher Π and C_{18}-bonded silica pre-columns. The Boos silica gives the cleanest chromatogram but has lost the two most volatile compounds.

Apart from the sorbents already mentioned, other sorbents such as Florisil (SiO_2 and MgO) can rapidly and efficiently recover PAHs for petroleum and sediment extracts as a clean-up step after another extraction technique.

Solid-phase extraction is widely used after the ultrasonication extraction or microwave extraction of soils and sediments, as a clean-up step before chromatographic determination. In these cases, Florisil or C_{18}-bonded silica are the sorbents which are most used. SPE is also used before supercritical fluid extraction to analyse aqueous samples and results are better with solid-phase extraction–supercritical fluid extraction than with supercritical fluid extraction alone. **Table 4** shows the results obtained when different extraction techniques were applied to determine PAHs in water samples. Much better results can be seen when solid-phase extraction technique is added to the supercritical fluid extraction one.

Most of the sorbents are used as cartridges but membrane discs can also be used to concentrate PAHs; the main advantages of discs are the considerable reduction in extraction time and the higher sur-

Table 4 Comparison of recoveries for different extraction techniques for extraction of 16 PAHs from spiked HPLC-grade water

PAH	SPE		SPE–SFE		SFE[a]		LLE	
	%Recovery	%RSD	%Recovery	%RSD	%Recovery	%RSD	%Recovery	%RSD
1	108	7	91	58	40	30	15	67
2	97	3	65	58	44	28	17	47
3	112	3	76	29	55	23	14	42
4	109	4	82	5	61	21	18	30
5	23	6	87	15	54	11	35	11
6	85	6	82	13	51	10	46	8
7	73	11	204	19	40	5	89	5
8	72	12	188	21	39	7	88	4
9	51	13	59	14	44	14	84	22
10	63	10	75	22	41	11	104	18
11	53	12	59	22	66	21	97	39
12	27	12	65	22	66	25	108	34
13	57	18	63	28	66	28	95	40
14	61	13	65	34	75	22	92	38
15	53	26	67	30	78	27	109	51
16	57	19	70	27	76	20	103	41

SPE, solid-phase extraction; SFE, supercritical fluid extraction; LLE, liquid-liquid extraction. Volatile PAHs:1, naphthalene; 2, acenaphthylene; 3, acenaphthene; 4, fluorene; 5, phenanthrene; 6, anthracene; 7, fluoranthene; 8, pyrene. High molecular PAHs: 9, benz(*a*)anthracene; 10, chrysene; 11, benzo(*b*)fluoranthene; 12, benzo(*k*)fluoranthene; 13, benzo(*a*)pyrene; 14, indeno(*1,2,3-cd*)pyrene; 15, dibenz(*a,h*)anthracene; 16, benzo(*ghi*)perylene.
[a]Direct SFE.
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face of extraction, which means fewer clogging problems for the sorbent. They also enable larger volumes to be extracted with the same amount of sorbent than when cartridges are used.

Two different membranes, C_{18}-bonded silica and SDB were studied to determine PAHs from surface water. Prior to the extraction procedure, the discs were conditioned to prevent interfering peaks in the chromatogram. In this process, for C_{18} discs, 20 mL of organic elution solvent (dichloromethane–ethyl acetate–acetonitrile, 50 : 30 : 20 v/v/v) was added to the filtration reservoir and slowly drawn through the discs by applying a slight vacuum. After drawing air through the discs for 5 min, 20 mL of an acetone–water mixture (80 : 20 v/v) was added and slowly drawn through the filtration discs, followed by 10 mL of Milli-Q water. The styrene-divinylbenzene copolymer discs were conditioned with 20 mL of acetone, 20 mL of acetonitrile and 20 mL of dichloromethane. After each addition a vacuum was applied to remove interfering compounds from the discs. Then, 20 mL of Milli-Q water was added and drawn through the disc before sample preconcentration.

Table 1 shows the recovery values obtained when C_{18}-bonded silica and SDB membrane discs were used in the determination of PAHs. In this case, 100 mL of Milli-Q water spiked with 0.5 μg L^{-1} of 15 PAHs and 29 μg L^{-1} of acenaphthylene were concentrated and then eluted twice with 15 mL of organic mixture. In this table the influence of adding 2-propanol to the sample is also shown, as already mentioned.

Another group studied the solid-phase extraction of PAHs in water samples using solid-phase extraction discs with a glass fibre matrix (GFM) and recoveries were good for the six PAHs studied. The main advantage of this GFM sorbent is the high speed of extraction, and the lower amount of solvent required compared with the conventional cartridge. **Figure 3** shows the chromatograms of analysis of seawater and riverwater using this kind of membrane.

Other selective sorbents, immunosorbents, based on antigen–antibody interactions, have also been used for selective isolation of PAHs from complex environmental samples. The extraction of the PAHs from sediments by sonication with dichloro-methane–methanol, subsequent extract dilution with water and extraction with an antifluorene immunosorbent gave similar results to those obtained with conventional clean-up procedures but selectivity was higher.

Desorption Solvent

After cleaning and conditioning the SPE cartridges or discs, the sample, with an organic solvent or micelle medium added, is applied. Then, organic solvents such as acetonitrile, tetrahydrofurane, acetone, *n*-hexane, methylene chloride, ethyl acetate and ethyl ether or mixtures of them are used to desorb PAHs.

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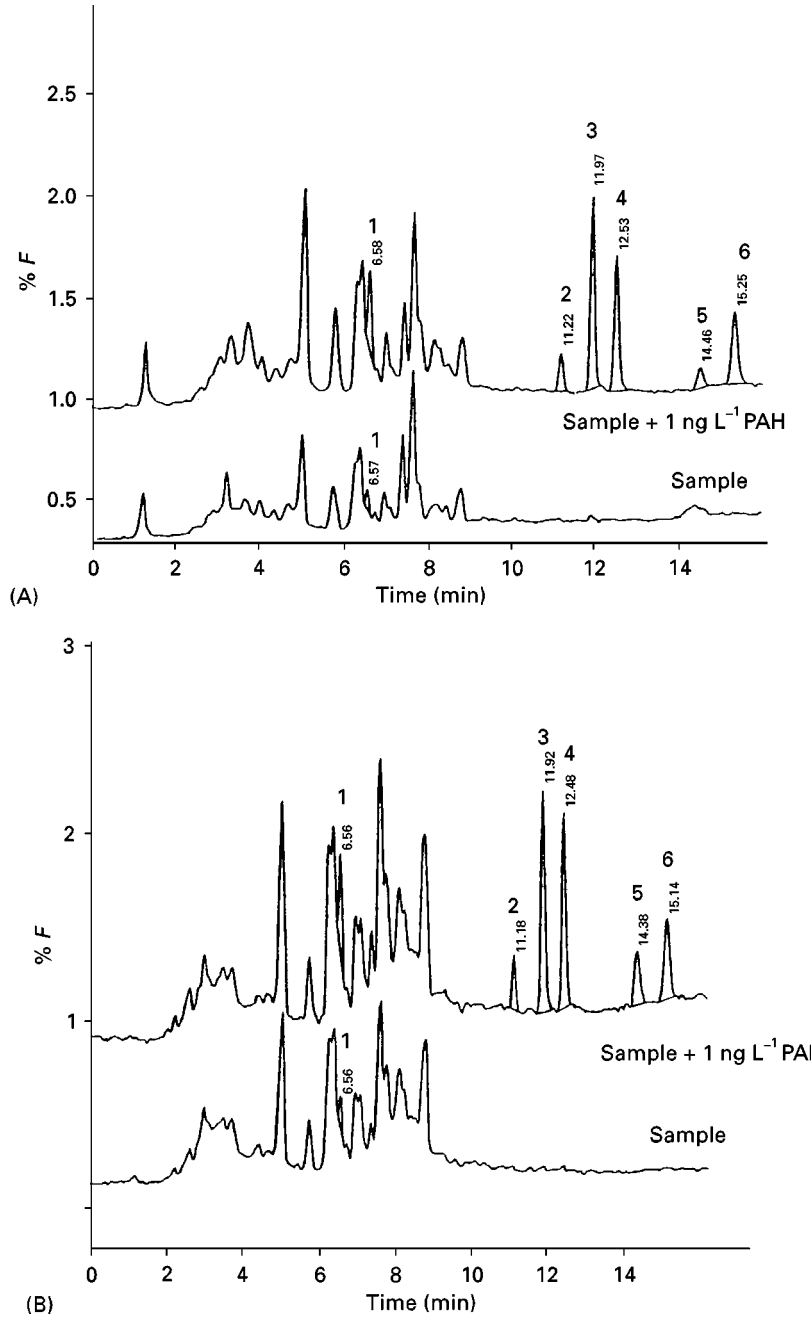

Figure 3 Separation and recovery of PAHs in (A) seawater and (B) riverwater, after being spiked with 1 ng L^{-1} for each compound, except fluoranthene (10 ng L^{-1}) and indenol[*1,2,3-cd*]pyrene (5 ng L^{-1}) to 1 L of sample. Peak assignation: 1, fluoranthene; 2, benzo[*a*]fluoranthene; 3, benzo[*k*]fluoranthene; 4, benzo[*a*]pyrene; 5, benzo[*ghi*]perylene; 6, indeno[*1,2,3-cd*]pyrene. Reproduced with permission from Urbe and Ruana (1997).

Recoveries of the low molecular weight PAHs are higher with nonvolatile solvents, but the recoveries for most of the PAHs with 4–6 aromatic rings are higher when a nonpolar solvent is used. So, in order to ensure optimum recoveries of all the PAHs, mixtures of solvents are usually recommended.

If the solvent used is compatible with the chromatographic system, concentration of the extracts is usually required to lower the limit of quantification. If the solvent is not compatible with the HPLC system, the extract obtained is dried with sodium sulfate evaporated at room temperature under a current of nitrogen or with a rotary evaporator and the residue dissolved in the mobile phase. The volume of organic solvent used in the solid-phase extraction membrane system is usually bigger than the volume

used in a cartridge system (between 30 and 50 mL and 1–5 mL, respectively).

Online Solid-Phase Extraction Chromatographic System

The main advantages of online systems are that the samples have to be manipulated less, they reduce analysis time and they consume less organic solvent.

SPE-HPLC is the most straightforward coupling method and therefore the most used. The sample preparation and conditioning steps are similar to those used in the offline but only the mobile phase is required to elute the compounds retained in the pre-column which is connected online to the analytical column.

The most used sorbents are the C_{18}-bonded silicas and SDB, although other specific sorbents can also be used. Depending on the sorbent and the solvent added to the aqueous sample, some interfering peaks can appear in the chromatogram, even when fluorescence detection is used. For instance, MeOH, acetonitrile and 2-propanol were tested as organic modifiers in the analysis of 50 mL of Milli-Q water with 15% of organic solvent by online solid-phase extraction with SDB discs. In this study, when using MeOH, peaks at the same retention time as naphthalene, phenanthrene, fluoranthene and pyrene appeared in the chromatogram. 2-Propanol gave peaks with the same retention time as naphthalene and phenanthrene. When acetonitrile was used, the blank was much cleaner and no interferences appeared in the chromatogram.

Table 5 shows the recoveries obtained when different percentages of acetonitrile are used in the analysis of PAHs by online SPE-HPLC. It can be seen that it is necessary to increase the percentage of acetonitrile to increase the recovery of high molecular weight compounds. However, this distorts the peaks of the low molecular weight compounds and so they could not be quantified. This happened to naphthalene, acenaphthene and fluorene when 25% of acetonitrile was used and, for this reason, 15% acetonitrile is the maximum concentration that can be used. However, at this percentage, the recovery value for the high molecular weight compounds is quite low, so the authors recommend that the PAHs should be analysed in two separate runs with 15% acetonitrile and 20% 2-propanol as modifiers, for the low molecular weight compounds (from naphthalene to pyrene) and the high molecular weight compounds (from benzo[*a*]anthracene to indeno[*1,2,3-cd*]pyrene). **Figure 4** shows a chromatogram of the analysis of 50 mL tap water using acetonitrile as organic solvent for low molecular weight compounds and IPA for the high molecular weight compounds.

Table 5 Recovery values obtained in the analysis of 50 mL of a standard solution of 0.4 μg L^{-1} using nine SDB discs at several percentages of ACN and IPA

Compounds	*Percentage of ACN*				*Percentages of IPA*		
	10%	*15%*	*25%*	*35%*	*10%*	*20%*	*30%*
Naphthalene	81	77	–	–	85	78	70
Acenaphthene	77	74	–	–	87	81	68
Fluorene	72	84	–	–	82	80	70
Phenanthrene	50	78	74	–	85	83	80
Anthracene	52	72	76	–	80	96	77
Fluoranthene	42	82	85	–	91	87	82
Pyrene	46	86	92	–	80	82	90
Benzo[*a*]anthracene	45	55	90	98	74	82	92
Chrysene	44	53	87	92	76	85	90
Benzo[*b*]fluoranthene	32	48	71	82	72	83	92
Benzo[*k*]fluoranthene	44	57	76	88	74	82	95
Benzo[*a*]pyrene	42	52	65	85	72	80	90
Dibenzo[*a,h*] anthracene	31	59	63	82	70	78	85
Benzo[*ghi*]perylene	28	58	69	78	68	82	89
Indeno[*1,2,3-cd*]pyrene	25	54	62	72	62	81	82

– Not quantified.
ACN, acetonitrile; IPA; isopropanol.

Using online SPE-HPLC it is possible to detect these compounds at very low ng L^{-1} levels. **Table 6** shows linearity range and limits of detection of PAHs in sea water.

Online coupling of solid-phase extraction to GC or SFC requires an additional step: drying the pre-column before elution with the organic solvent in order to remove the water present when aqueous samples are analysed. An organic solvent added to the samples is also required in order to avoid adsorption of the PAHs. When SPE-GC is used, a small volume of an organic solvent, usually ethyl acetate, is used to pass the retained compounds to the chromatographic column via a loop type or an on-column interface. When SPE-SFC is used, the same mobile phase is used to elute the retained compounds.

Conclusions

PAHs are mainly determined by HPLC fluorescence and detection because of its high sensitivity. To reach the very low levels of concentration required, an extraction concentration technique is necessary. Solid-phase extraction is the preferred technique both for extracting aqueous samples and cleaning up after other extraction techniques.

C_{18} silica cartridges or discs are the most recommended in online and offline modes.

One of the characteristics of the extraction of PAHs is the need for an organic solvent or a sufactant

DropBooks

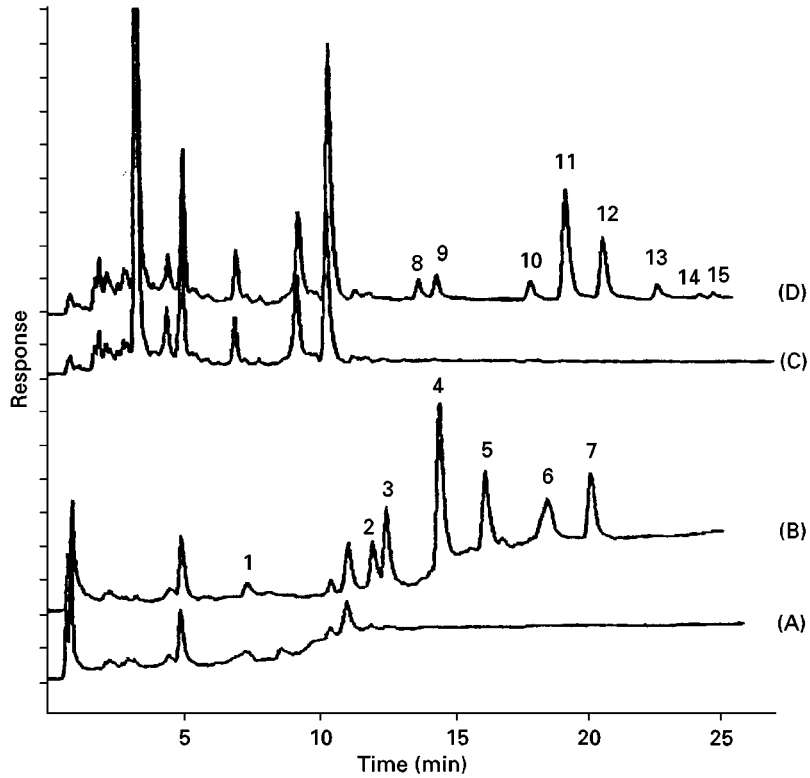

Figure 4 LC chromatogram of the analysis of 50 mL tap water using acetonitrile as organic solvent for low molecular weight compounds and isopropanol for the high molecular weight compounds. Analysis of low molecular weight compounds in tap water with addition of a standard solution of 10 ng L^{-1} (B) and without any addition (A). Analysis of high molecular weight compounds in tap water in the same conditions (C,D). Peak assignation: 1, naphthalene; 2, acenaphthene; 3, fluorene; 4, phenanthrene; 5, anthracene; 6, fluoranthene; 7, pyrene; 8, benzo[*a*]anthracene; 9, chrysene; 10, benzo[*b*]fluoranthene; 11, benzo[*k*]fluoranthene; 12, benzo[*a*]pyrene; 13, dibenz[*a,h*]anthracene; 14, benzo[*ghi*]perylene; 15, indeno[*1,2,3-cd*]pyrene. (Reproduced with permission from El Harrak *et al.* (1998)).

to be added to the samples, in order to avoid adsorption of these compounds in the system.

The use of solid-phase extraction and HPLC with fluorescence detection enables low levels (at ng L^{-1} in water samples) of PAHs to be determined in real samples.

Table 6 Linear range and limits of detection of PAHs in seawater. Volume of sample 50 mL

Compound	*Linear range (ng L^{-1})*	r^2	*LOD (ng L^{-1})*
Naphthalene	400–2.0	0.9994	1.0
Acenaphthene	400–1.0	0.9997	0.5
Fluorene	400–1.0	0.9995	0.5
Phenanthrene	400–0.5	0.9999	0.2
Anthracene	400–1.0	0.9998	0.5
Fluoranthene	400–1.0	0.9997	0.5
Pyrene	400–0.5	0.9998	0.2
Benzo[*a*]anthracene	200–2.0	0.9997	0.5
Chrysene	200–2.0	0.9999	0.4
Benzo[*b*]fluoranthene	200–2.0	0.9999	0.3
Benzo[*k*]fluoranthene	200–0.5	0.9998	0.2
Benzo[*a*]pyrene	200–2.0	0.9997	0.2
Dibenzo[*a,h*]anthracene	200–0.5	0.9998	0.2
Benzo[*ghi*]perylene	200–5.0	0.9996	2.0
Indeno[*1,2,3-cd*]pyrene	200–5.0	0.9995	2.0

See also: **III/Environmental Applications:** Solid-Phase Microextraction; Supercritical Fluid Extraction. **Immunoaffinity Extraction: Polycyclic Aromatic Hydrocarbons**; Gas Chromatography; Supercritical Fluid Chromatography; Thin-Layer (Planar) Chromatography.

Further Reading

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Supercritical Fluid Chromatography

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Introduction

There is tremendous analytical interest in polycyclic aromatic compounds (PAC) because of their carcinogenic properties and their impact on the environment. PAC mixtures can be very complex and extend over a wide range of molecular masses. They mainly originate from the combustion of fossil fuels and are widespread in the environment.

The most commonly used separation techniques for PAC are gas chromatography (GC), high performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC). GC is often the technique of choice since it works well for low molecular weight and volatile compounds. It is also preferred because the high diffusivity of solutes in gases results in relatively short analysis times. HPLC employs liquids of high solvating power, high densities and high viscosities but is often time-consuming and has much less resolving power than GC.

Separation of PAC by SFC

Although HPLC and GC are commonly used for the analysis of PAC mixtures, SFC has a number of advantages. SCF have diffusivities that are more gas-like, viscosities lower than liquids and densities that are more liquid-like. The resulting mass transfer coefficients lead to a more rapid analysis in SFC than in HPLC. The diffusion coefficients and viscosities of SCF allow GC-like separations on capillary columns but at much lower temperatures because of the high solvating power of SCF. The approach in SFC involves the use of columns of the packed LC type, packed capillary (micro-packed) and the open tubular (capillary) GC type columns.

SFC on Packed Columns

Packed columns usually of the commercial HPLC variety were used in nearly all the early work in SFC. The length and internal diameter (i.d.) of SFC packed columns are constrained by (a) the larger pressure drops as compared to open tubular columns and (b) high flow rates, making interfacing to GC detectors more difficult. Often, narrow-bore packed columns with a diameter of 1 to 2 mm are used because they can be installed in a capillary SFC instrument and are compatible with many detectors.

Early work on the separation of polycyclic aromatic hydrocarbon (PAH) standards revealed that reduced particle size led to increased resolution, and that analysis times were reduced in comparison with HPLC; it was also shown that PAH elution order could be varied by changing the operating temperature and/or the pressure. Marked changes in retention and selectivity are brought about by the addition of a modifier to the mobile phase. Detailed studies of the retention characteristics of PAH on a wide range of packed columns showed that retention in SFC with CO_2-based mobile phases correlates most closely with reverse phase HPLC. Very rapid analysis (< 6 minutes) of the sixteen Environmental Protection Agency target PAH was demonstrated on a 15 cm long column packed with a specially bonded C_{18} silica (**Figure 1**). Efficient separation of PAH can be achieved by coupling columns of different selectivity. Plate numbers up to 220,000 and separation of up to